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Please find below and/or attached an Office communication concerning this application or proceeding.

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ATTACHMENT

Applicant's Remarks filed on 9/15/2010, after the final action, have been fully considered and are fully addressed below. Upon entry of Applicants' After-final Amendment to the Claims filed on 9/15/2010, for purposes of appeal, the claims stand rejected as follows:

Claim Rejections - 35 USC § 102-maintained

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

The rejection of Claims 4-6, 10-14, 16-18 and 22, under 35 U.S.C. 102(b) as being anticipated by Hinds et al in "Enhanced gene replacement in mycobacteria" (Microbiology, 1999, Vol. 145: p. 519-527, entire document; of record) is maintained for reasons of record and presented herein.

Applicants arguments have been fully considered but are unpersuasive.

The applicant traverses the rejection and argues that the currently amended claim 4 is not anticipated by the cited prior art reference stating:

> The present application relates to the finding that a replication competent DNA molecule treated with a mutagenic substance that blocks its replication, when transformed into a prokaryotic or eukaryotic cell, undergoes much higher levels of homologous recombination than an untreated DNA molecule. Thus, the treated DNA molecule is a far superior agent for transforming prokaryotic or eukaryotic cells by targeted homologous recombination. This method overcomes some of the limitations associated with previous methods of transforming a prokaryotic or eukaryotic cell by targeted homologous recombination.
>
> As described in the present specification, the increased level of homologous recombination is likely due to the generation of highly "recombinogenic" ends resulting

from partial replication of the treated DNA molecule in the target cell. These highly recombinogenic ends remain active as the replication of the DNA molecule cannot become complete due to the treatment of the DNA molecule with the mutagenic/replication blocking substance.

As such, claim 4 is directed to a method for in vitro insertion of a nucleic acid of interest within a predetermined target nucleotide sequence present in a chromosome. The method includes in part:

a) providing a DNA vector that is replication competent in the target cell comprising the nucleic acid of interest and contacting the DNA vector with a mutagenic agent blocking intracellular DNA replication of the DNA vector to produce a modified DNA vector;

b) transfecting the target cells with the modified DNA vector obtained under conditions wherein replication of the modified DNA vector commences and insertion of the nucleic acid of interest within the predetermined target nucleotide sequence occurs; and

c) selecting cells where the nucleic acid of interest has been integrated into the predetermined target nucleotide sequence.

HINDS fails to teach or suggest such a method. HINDS describes a method for homologous recombination (HR) in Mycobacterium that uses plasmid vectors treated with UV radiation, or so called "suicide vectors." In contrast to the present claimed method, these suicide vectors are not replication competent and do not replicate in the target cells (mycobacteria).

The Office Action appears to misinterpret the methodology of HINDS. In particular, the recombination assays using the pRAM4 shuttle vector are based upon an intraplasmid recombination event and do not lead to the introduction of a sequence at a target in the host cell genome. Indeed, what the authors in HINDS are looking for when using the pRAM4 vector is the loss or retention of a kanamycin resistance cassette positioned between two parts of a hygromycin resistance cassette. This means that the non-recombined pRAM4 vector will give rise to kanamycin resistant/hygromycin sensitive colonies and the recombined pRAM4 vector will give rise to kanamycin sensitive/hygromycin resistant colonies.

Again, the method of present claim 4 features the insertion of a nucleic acid of interest initially included in a DNA vector within a predetermined target nucleotide sequence in the genome of a target cell. In distinction, the HINDS recombination assays in Mycobacterium using pRAM4 were performed "To identify experimental conditions for the application of gene replacement experiments that favoured HR, a plasmid-based recombination assay was developed which would generate quantitative results. The recombination assay vector, pRAM4, was constructed based upon restoration of a functional hyg gene by an HR event between two overlapping fragments of hyg gene

flanking a functional kan gene in a shuttle plasmid (Fig. la, b Intraplasmid HR between the duplicated DNA would restore a complete and functional hyg gene, deleting the kan cassette in the process" (emphasis added), (see, page 521, column 2, paragraph 2). Thus, HINDS discloses an intraplasmid HR assay, i.e., an assay of HR events within the same plasmid, using the pRAM4 shuttle vector. No insertion of any sequence contained within the pRAM4 shuttle vector into the host cell genome occurs or is tested using this assay.

Also, the Office Action's contention that the pRAM4 vector is treated with a mutagen that prevents replication of this vector is not correct. In fact, it is the presence or absence of the replicated progeny of the original or recombined pRAM4 shuttle vector in the later generations of the target cell that allows the authors to measure the effects of the various mutagens on intra-pRAM4 vector recombination. HINDS fails to teach or suggest the use of anything other than a suicide vector for the introduction of a DNA sequence by HR into a target cell genome. HINDS also does not teach that a replication competent vector would be used for such a purpose.

Applicants arguments have been fully considered but are unpersuasive. Hinds et al teach a method comprising contacting a DNA vector (i.e. the replication competent pRAM4 vector) with a mutagenic agent (i.e. UV-irradiation) blocking intracellular DNA replication of the DNA vector to produce a modified vector prior to transformation of the modified vector into target cells and then transfecting/transforming the target cells with the modified of bacterial plasmid vector DNA in order to enhance subsequent homologous recombination in the mycobacteria (e.g. page 521, right column, last paragraph and page 523 Figure 2 and legend). Hinds et al teach the inactivation of *M. smegmatis* genes and the use of a recombination assay to identify conditions whereby mutagenic agents (i.e. UV irradiation) enhance homologous recombination. Thus, contrary to the applicants assertion that because Hinds et al teach non-replicating suicide vectors and ss phage vectors that the reference therefore does not anticipate the instantly amended claims is not persuasive because, as discussed above, in addition to those non-replication competent vectors, Hinds et al also teaches methods using replication competent vectors and teaches UV treatment prior to transfection of target cells using conditions where

replication commences. Thus, Hinds et al anticipates all the limitations of claims 4-6, 10-14, 16-18 and 22.

Therefore, Claims 4-6, 10-14, 16-18, and 22 stand rejected under 35 U.S.C. 102(b) as being anticipated by Hinds et al for reasons of record and above.

Claim Rejections - 35 USC § 103-maintained

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 4-21 and 23-24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hoeijmakers et al in "Detection Methods Based on HR23 Protein Binding Molecules (US PGPub

No:2003/0124605, filed 20 November 2002, which claims priority to Provisional Application No:60/331.773, filed 21 November 2001, see entire document; of record) in view of Hinds et al (above).

Applicants arguments have been fully considered but are unpersuasive. The applicant traverses the rejection and argues that the currently amended claim 4 is not anticipated by the cited Hinds prior art reference stating:

> First, as detailed in the above remarks, HINDS fails to teach or suggest any method having the combination of features as recited in the present claims.

In addition Applicant argues:

> Second, the Office Action appears to have misinterpreted HOEIJMAKERS. HOEIJMAKERS relates to methods to monitor the levels of HR23 protein binding partners in cells following exposure to a DNA modifying agent (i.e., a mutagen). The HR23 protein binding partners investigated include XPC, MAG, CREB, and p53. HR23 and its binding partners are one of the in vivo mechanisms involved in repairing DNA lesions in the host cell genome.
>
> The cells used in this method were engineered so as to have altered expression of HR23A and/or HR23B. For HR23A, this was achieved by targeted gene disruption in accordance with the scheme shown in Figure i. The strategy outlined in Figure 1 and detailed at page 9, paragraphs 108-115, is a known homologous recombination gene alteration strategy. This strategy involves the generation of a mHR23A targeting vector which has two portions of homology to exons II and exons VII/VIII of the HR23A gene and flanking a neomycin resistance cassette (see, Figure 1 and paragraph [0108]). This non-replication competent targeting vector (it is a bacterial plasmid and the target is a mammalian cell) is then electroporated into the target cells. Following culturing and expansion of the initial target population, G418 resistant clones are selected based upon the presence of the neomycin resistance cassette as a stable integrant. This is verified by way of two restriction enzyme digests of genomic DNA from the selected clones to verify that the clones contained the neomycin resistance cassette in the correct position in the genome disrupting the HR23A gene (see, Figure 1 and paragraph [0110]).
>
> Using these transformed cells, transgenic mice and immortalized cell lines were created which had a homozygous HR23A ! genotype. The HR23A ! mice were interbred with existing HR23B ! mice (Ng et al., Mol. Cell Biol. 22, 1233-1245, 2002) to produce homozygous double mutant HR23A ! B ! mice (see, paragraph [0112]).

For HR23B, a full length cDNA of this gene was inserted into the pSLM vector and then transfected into DKO MEF (Double- Knockout Mouse embryo fibroblasts) cells from the mice bred in paragraph [0112], together with a separate vector comprising a full length cDNA of the hXPC-GFP fusion protein. The resulting transformed cells were then selected for stable transformants of these transgenes based upon the presence of the puromycin resistance cassette present in the vectors (see, paragraphs [0129] and [0130]). The vectors used in these experiments are not replication competent, they randomly insert themselves into the target cell genome as per the normal transfection protocol.

Therefore, HOEIJMAKERS details two methods of transforming a target cell population:
1) The method used to knock-out the HR23A gene, by inserting via a homologous recombination event a neomycin resistance gene which is inserted in place of several exons of the HR23A gene removing its function; and
2) The method used to insert (knock-in) the HR23B and hXPC-GFP transgenes using transfection vectors and the selection of stable transformants again on the basis of the insertion of the neomycin resistance gene into the host cell genome.

Only one of these methods uses homologous recombination as the means of transforming the host cell (Method i) and in this transformation method it is noted that the J'mHR23A Targeting Vector" is not capable of replicating in the target cell (because it is a bacterial plasmid and the target cell is mammalian). Also, at no point prior to or following the introduction of this targeting vector into the target cell was it exposed to any mutagen such as UV or a chemical mutagen. Such exposure only occurred after the stable clone resulting from the HR event had been selected for and isolated. HOEIJMAKERS prepared their panel of HR23A/B knock-out / knock-in mouse cell lines so that the effects of mutagens on these cell lines could be studied. HOEIJMAKERS is not concerned with, and fails to teach or suggest anything about, methods of transforming a cell so as to introduce a nucleic acid sequence of interest other than using standard known techniques such as by way transfecting a cell and selecting for transformants (method 2) or introducing a non-replication competent DNA targeting vector with homologous portions to those of a target genomic locus, which following a rare homologous recombination event leads to the insertion of a selectable marker and some change in the locus which is then selected for based upon the insertion of a selectable resistance cassette. The subsequent treatment of these HR23A and/or HR23B knock-out/knock-in cell lines (what the Office Action refers to as J'cells stably expressing hXPC- GFP/hHR23B were rinsed with PBS, exposed to UV... and subsequently cultured at 37°C for various time periods...") refers to the exposure of stably transformed cell lines which comprise the hXPC-GFP/hHR23B transgenes stably chromosomally integrated, with the specified mutagen so that the effects of this in the engineered genetic background can be assessed.

> This exposure of the cells to a mutagen is therefore not so that these already stably integrated transgenes can become integrated as the Office Action suggests. Thus, HOEIJMAKERS teaches the same J'standard" strategy for performing a genetic transformation event using homologous recombination, that is, the homologous recombination vector is a suicide vector (it cannot replicate in the target cell) so that transformants which result from homologous recombination can be selected without a massive background of the selection cassette being present as a non-genomically integrated plasmid (or other genetic structure) in the target cell.

Thus, Applicants' conclude:

> For all of these reasons, the combination of HOEIJMAKERS and HINDS fails to teach or suggest, and fails to render obvious, the methods of present claims 4-21 and 23-24.

Applicants arguments have been fully considered but are unpersuasive. Hoeijmakers et al teach a method of targeted homologous recombination using vectors comprising identical 5'- and 3'- sequences respective to the target DNA contained in the chromosome (see especially Figure 1). Hoeijmakers et al teach the use of the mutagenic agents such as UV irradiation and 50 and 100uM concentrations of N-acetoxy-2-acetylaminofluorene (NS-AAF) (p.10, ¶ 132) and wherein the nucleic acid of interest encodes a protein of therapeutic interest, wherein an open reading frame is disrupted by a heterologous nucleotide sequence, and which codes an antisense RNA. For example, Hoeijmakers et al recite:

> An Ola129 mHR23A targeting construct was generated by converting the BglII site in exon II of clone pG7M23Ag1 (containing a 4 kb genomic EcoRI fragment subcloned in pGEM7) into a ClaI site, which (due to a ClaI site in the polylinker) allowed deletion of sequences downstream of the BglII site in exon II (clone pG7M23Ag7). Next, the remaining EcoRI site was removed by filling-in the overhangs with Klenow, resulting in clone pG7M23Ag9. After changing the BstXI site into a SalI site, the 3 kb XhoI-SalI fragment was cloned into SalI digested pGEM5, resulting in clone pG5M23Ag17. Next, the 3' arm of the construct, consisting of a Klenow-blunted 1.5 kb SmaI-XbaI fragment starting at the SmaI site in exon VII, was inserted in the blunted NdeI site of pG5M23Ag17 (giving pG5M23Ag20), followed by insertion of a Neo marker cassette in antisense orientation in the ClaI site (giving pG5M23Ag24). Finally, the NotI-NsiI insert of pG5M23Ag24 was recloned into a pGEM-9Zf(-) based vector containing a 2.8 kb thymidine kinase (TK) marker cassette (giving pG5M23Ag30).

In addition, Hoeijmakers et al teach that "cells stably expressing hXPC-GFP/hHR23B were rinsed with PBS, exposed to UV-C light (254 nm; Philips TUV lamp, dose as indicated in the text) and subsequently cultured at 37°C for various time periods (as indicated in the text). XPC was detected either by immunoblot analysis or by visualization in living cells using fluorescence microscopy. A similar approach was used to study the effect of N-acetoxy-2-acetylaminofluorene (NA-AAF, final concentration 50 or 100 μM)"(p.10, ¶ 132), and further teaches mouse and human (HeLa) cells (p.11, ¶ 136 and 141).

However, Hoeijmakers et al fail to teach wherein the DNA vector is treated with the UV prior to transfection into the target cells.

Hinds et al teach methods of homologous recombination and show positive results treating DNA vectors with a mutagenic agent (i.e.UV) prior to transfection into target cells. For example, Hinds et al teach a method comprising contacting a DNA vector (i.e. the replication competent pRAM4 vector) with a mutagenic agent (i.e. UV-irradiation) blocking intracellular DNA replication of the DNA vector to produce a modified vector prior to transformation of the modified vector into target cells and then transfecting/transforming the target cells with the modified of bacterial plasmid vector DNA in order to enhance subsequent homologous recombination in the mycobacteria (e.g. page 521, right column, last paragraph and page 523 Figure 2 and legend). Hinds et al teach the inactivation of *M. smegmatis* genes and the use of a recombination assay to identify conditions whereby mutagenic agents (i.e. UV irradiation) enhance homologous recombination.

It would have been obvious to one of ordinary skill in the art to combine the method of Hoeijmakers et al with the step of Hinds et al and try contacting the DNA vectors used in

methods of homologous recombination of Hoeijmakers et al with the mutagenic agent prior to transfection into the cell because Hinds et al show that this method was successful. One of ordinary skill in the art would have been motivated to do so because Hinds et al show that treating the DNA vectors prior to transfection yielded improved results when compared to treatment in the cell (e.g. page 521, right column, last paragraph and page 523 Figure 2 and legend).

Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result when utilizing the method of prior mutagen treatment (as taught by Hinds et al) in the homologous recombination methods of Hoeijmakers et al.

In view of the foregoing, the method of claims 4-21 and 23-24, as a whole, would have been obvious to one of ordinary skill in the art at the time the invention was made. Therefore, the claims are properly rejected under 35 USC *§103(a)*.

Claim Rejections - 35 USC § 112-maintained

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 4-24 are rejected under 35 U.S.C. 112, **second paragraph**, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicant's arguments have been fully considered but are unpersuasive. Applicant argues:

Currently amended claim 4 further clarifies the intended subject matter and addresses the issues noted in the Office Action. Accordingly, Applicants request reconsideration and withdrawal of the rejection.

Applicant's argument is unpersuasive because the current amendments to claim 4 consist of substituting the term "contacting" with the term "providing" in line 7 and inserting the phrase ", and contacting said DNA vector" in line 9-10. Applicant has provided no reasoning as to how these amendment clarify the intended subject matter. Contrary to applicants arguments, currently amended independent claim 4 remains unclear as to what is encompassed regarding the active method step of part (a) which now recites "providing a DNA vector that is replication competent in said target prokaryotic or eukaryotic cell and comprises the nucleic acid of interest, and contacting said DNA vector with a mutagenic agent blocking intracellular DNA replication of said DNA vector, to produce a modified DNA vector" because it remains unclear whether the active step of contacting a DNA vector with a mutagenic agent must or should occur within a cell or whether the treatment with the mutagenic agent should or could occur outside of a cell. The claim is still missing a method step of contacting a cell with the aforementioned DNA vector before modification with the mutagenic agent but the phrase "with a mutagenic agent blocking intracellular DNA replication of said DNA vector" suggests the DNA vector is intended to be contacted with the agent in the cell. Therefore, one of ordinary skill in the art would not be able to ascertain the metes and bounds of applicant's invention.

Claims 5-24 are indefinite insofar as they depend from claim 4.